The study of solution equilibria in chiral capillary electrophoresis by the ligand-exchange mechanism

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In chiral separation, capillary electrophoresis (CE) is a leading technique and, due to the absence of any stationary phase, it is an all-in-solution process, thus allowing accurate study of separation mechanisms. This review focuses on the leading role played by knowledge of solution equilibria for a deep understanding of the phenomena involved in the chiral-discrimination mechanisms at a molecular level in chiral ligand-exchange CE.

Speciation diagrams have been accurately calculated on the basis of the stability-constant values reported in the literature for the evaluation of the species present in solution and their concentrations. This analytical approach turned out to be very useful in understanding electrophoretic behavior and should be used systematically to predict and to optimize chiral separations.

Keywords: Capillary electrophoresis (CE); Chiral separation; Cyclodextrin; Electromigration; Equilibrium constant; Ligand exchange; Metal complex; Separation; Solution equilibrium; Speciation diagram

Abbreviations: AA, Amino acid; AHNS, Amino-hydroxy-naphthalene-disulfonate; ANA, Amino-naphthalene-sulfonate; ANTS, 8-aminonaphthalene-1,3,6-trisulfonate; AP, Amino pyridinyl; BGE, Background electrolyte; BIN, Bis-indole; CE, Capillary electrophoresis; CEC, Capillary electrochromatography; CSP, Chiral stationary phase; CZE, Capillary zone electrophoresis; Dns, Dansyl; EKC, Electrokinetic chromatography; EMO, Electro migration order; FMOC, Fluorenyl-methyl-oxy-carbonyl; HA, Hydroxy acid; HPLC, High-performance liquid chromatography; LECE, Ligand-exchange capillary electrophoresis; MEEKC, Micro-emulsion electrokinetic chromatography; MEKC, Micellar electrokinetic chromatography; MS, Mass spectrometry; NBD, Nitro-benzo-diazole; PMP, 1-phenyl-3-methyl-5-pyrazolone; SID, Single-isomer derivative

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1. Introduction

Enantiomeric separation is difficult, since two molecules, otherwise equal, must be separated on the basis of just a slight structural difference, the spatial disposition of the same groups. For this, formation of diastereoisomeric complexes between the two enantiomers of a racemate and a chiral selector has been widely exploited. In this field of chiral separations, several techniques have been developed and several reviews published [1,2].

Direct enantioseparation by high-performance liquid chromatography (HPLC) has significantly advanced, and a large number of chiral stationary phases (CSPs) for HPLC have been developed using both chiral small molecules and polymers with chiral-recognition abilities [3,4]. Furthermore chiral separations have also been fulfilled in HPLC by chiral mobile phase [5], gas chromatography (GC) [6], supercritical fluid chromatography (SFC) [6,7] and planar chromatography (PC) [8,9]. Electromigration techniques [10,11] {capillary electrophoresis (CE) [i.e. capillary-zone electrophoresis (CZE) or electrokinetic chromatography (EKC)], micellar electrokinetic chromatography (MEKC), microemulsion electrokinetic chromatography (MEEKC), capillary electrochromatography (CEC), and microchip CE} have been widely exploited in chiral separation due to their versatility, high speed, sensitivity and low running costs.

In every case, the process responsible for the separation is the formation of diastereoisomeric complexes between the enantiomers and a chiral selector. However, with the exception of CE, for all the above techniques, we are dealing with homogeneous equilibria occurring in the mobile phase and the additional heterogeneous ''quasi equilibria'' occurring when the analytes interact with the stationary or

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pseudo-stationary phase. In CE only, as well stated previously [12–14], the separation takes place without the presence of any heterogeneous phase giving rise to ''an all-in-solution process'' that can be accurately characterized in advance, by identifying the species in solution and determining their equilibrium constants. Investigation of the chemical reactions cannot be based on the stoichiometry only: many reactions are reversible, so they result in an equilibrium mixture of reactants and products. Consequently, if species A and B coexist in solution and they react to form one or more complexes of general formula B_qA_p, they do not automatically form the species corresponding to the ratio of their analytical concentrations. The species and their relative concentrations strictly depend on the number and the stoichiometry of the species themselves and on the values of their stability constants. If all the stability constants for a given system have been determined, it is possible, in principle, to calculate the concentration of each of the species present under a known set of experimental conditions. Such exact knowledge of the composition of a solution can be used to predict the conditions required for the optimal formation of a given complex, giving great importance in planning separation procedures and dramatically improving their performances.

As described above, in CE, there is no heterogeneous phase, so the heart of the separation process is the formation of the diastereoisomeric complexes between the enantiomeric analytes and a chiral molecule or a metal complexed by a chiral ligand. In this latter case, the technique is called ligand-exchange CE (LECE), as it invokes ligand exchange between the second ligand or the water in the coordination sphere of the metal ion [15,16].

Every chemical reaction proceeds with a specific velocity (i.e. it has its own kinetics). Obviously, to conduct separations properly based on complexation reactions, these last should occur quickly, so the complexes must be labile. In such kinds of complex, the bond(s) between the ligands or between the ligands and the metal ions are formed and broken continually. In this case, the thermodynamic stability of the complexes can be conveniently expressed as the mean time spent by the ligand molecules bound to the metal ion or the other ligand. In these conditions, the stability of the complexes and the degree of affinity of the ligands are critical. If the affinity is too low, regardless of the lability of the complexes, the ligands will spend a negligible fraction of time bonded to the metal ion. Although occurring, this process will therefore have only a slight effect on the ligand behavior. However, too high a degree of formation of the complexes gives rise to the opposite effect, and removes any difference between the analytes.

Furthermore, the conditions to be fulfilled in CE in order to obtain successful separation of the investigated enantiomeric analytes are:

(1) the analytes give rise to complexes in solution with a significant degree of formation;
(2) the formation rate of the complexes is high (labile complexes);
(3) the degree of formation of the complexes is significantly different between the two enantiomeric analytes; and,
(4) the complexes formed have electrophoretic mobilities different from those of the free ligand.

Even though full characterization of the complexation equilibria occurring in solution is crucial to achieve a rational design of separation experiments, this approach has not been used extensively in the literature. The role of so-called secondary equilibria has been outlined in many papers [12–14,17,18], but no one has utilized the thermodynamic stability constants (independently determined) and carried out accurate simulations to explain what is really going on in solution. In particular, if chiral separations exploit the formation of metal complexes [19], as in LECE, the solution equilibria involved are well known in numerous cases.

This review covers the developments in chiral LECE from late 1980s to March 2011, in retrospect explaining, if possible, the results obtained by analysis of the equilibria occurring in solution, excluding the papers dealing with LE-MEKC and non-aqueous solvents. Although MEKC shows several advantages, the introduction of surfactants and the consequent formation of micelles would imply the presence in solution of a pseudostationary phase, and this would lead to discussion on heterogeneous quasi-equilibria that are outside the topic of this review. In the same way, non-aqueous systems have not been considered due to the limited data on equilibrium in the literature.

2. Investigation of solution equilibria

Several solution properties vary in a measurable extent as an effect of complex formation. Hence, stability constants may be determined by various techniques, provided that the effects of complex formation quantitatively affect the properties of the solutions. Thus, the choice of an adequate method is an essential prerequisite to obtain reliable values for stability constants.

Any method that may be used to determine with reasonable accuracy the concentration of at least one of the species in solution provides the information needed, together with the known analytical concentrations, to calculate the concentrations of all the remaining species present in solution and hence the equilibrium constants. If a sufficient number of such equilibrium measurements are carried out over a sufficient range of experimental conditions, we can obtain accurate values of stability constants that apply within the range of reaction conditions employed.